ViroGene HCV Quantitative Kit

Real Time PCR Quantitative Kit

Article No 721012, 721112, 721212 CE IVD

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ViroGene HCV RT-PCR Kit 1.0

Real-Time RT-PCR Detection

A. General

- ViroGene® HCV RT-PCR Kit 1.0 is an in vitro diagnostic test, based on real-time PCR technology, for the detection and quantification of human hepatitis C virus (HCV) specific RNA (genotypes 1 to 8) in human Serum and EDTA plasma.
- The Quantitative testing with ViroGene® HCV RT-PCR Kit kits is based on a duplex Real-Time RT-PCR: In one reaction setting, the RNA target sequences for HCV as well as for the Exogenous control are reverse transcribed (Reverse Transcription (RT)) and amplified in parallel with respective primer and probe pairs in the Polymerase Chain Reaction (PCR). Amplified target gene fragments are detected via fluorescently labeled probes during the PCR reaction in real-time (Real-Time PCR). The probes specific for detection of amplified HCV RT-PCR Kit (5`UTR) and the Exogenous control target genes are labeled with fluorescent dyes FAM and HEX, respectively, and their emitted fluorescence is separately optically measured by the Real-Time PCR thermal cycler. By means of both individual analyses in one reaction vessel per sample and the Negative Control and Positive Control per run the HCV specific status of a sample can be evaluated in the end. This way, results can be achieved within a few hours after sample receipt.
- These kits were developed for use by trained laboratory personnel following standardized procedures. This Direction For Use must be followed strictly. For in vitro diagnostic use only.
- The ViroGene Quantitative HCV RT-PCR kit can be used with the following real-time

PCR instruments:

Applied Biosystems 7300 / 7500 Real-Time PCR System

AriaMx Real-Time PCR System

CFX Connect™ / CFX96™ / Dx Real-Time PCR Detection System

DTlite Real-Time PCR System

LightCycler® 2.0 / 480

QuantStudio™ 3 Real-Time PCR System

Rotor-Gene 3000 / 6000 / Q

SLAN® Real-Time PCR System

B. Reagents and Materials

- The following ViroGene® HCV Quantitative kits are available and comprise the following reagents:

Reagent	100 Reactions Article No 721012 REF	500 Reactions Article No 721112 REF	Concentration	Store at
RNA Buffer Mix	1 x 1250 µl	5 x 1250 µl		≤ -18 °C
RT Enzyme Mix	1 x 100 µl	5 x 100 µl		
Quantification Standard 1	1 x 100 µl	1 x 100 µl	3x10^7	
Quantification Standard 2	1 x 100 µl	1 x 100 µl	3x10^6	
Quantification Standard 3	1 x 100 µl	1 x 100 µl	3x10^5	
Quantification Standard 4	1 x 100 µl	1 x 100 µl	3x10^4	
Primer Probe Mix	1 x 150 µl	5 x 150 µl		
Internal Control	1 x 1000 µl	5 x 1000 µl		

- After receipt, the components are immediately stored at ≤ -18 °C. Avoid repeated freezing and thawing of all the reagents and keep them thawed as short as possible. If occasional processing of few samples only is expected you may prepare appropriate aliquots of reagents before storage at ≤ -18 °C. Prepare aliquots in such a way that freeze-thaw-cycles are reduced to a maximum of three. The Negative Control can alternatively be stored at +2°C to +8°C.
- The components are to be used within the indicated shelf life (see box label). The components of different batches may not be mixed.
- The Reaction-Mix needs to be stored protected from abundant light. Do not expose to direct (sun)light.

C. Equipment and Reagents not included

- This detection method can be used on all commercially available Real-Time PCR thermal cyclers that detect the emitted fluorescence of the fluorescent dyes FAM and HEX (emission 520 and 550 nm, respectively). Note that default normalization option against ROX (e.g. using ABI cyclers) must be deactivated.
- Apart from the disposables, the following further devices are needed and are not included in the ViroGene® HCV Quantitative kits:

  RNA preparation kit / protocol

(e.g. ViroGene® RNA / DNA Purification products)
Table top microcentrifuge
Vortex
Micropipettes covering volumes of 1 µl to 1000 µl
Centrifuge for PCR tubes or plates

- We recommend the exclusive use of certified Nuclease-free disposables as well as powder-free protective gloves. Please wear gloves during the entire experimental procedure. Gloves need to be changed frequently, especially after spillage or suspected contaminations.

D. Sample Preparation

- Extracted RNA is the starting material for ViroGene Quantitative HCV RT-PCR kit the quality of the extracted RNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

The following nucleic acid extraction systems and kits are recommended:

VERSANT™ Molecular System SP (Siemens)

HighPure® Viral Nucleic Acid Kit (Roche)

QIAamp® Viral RNA Mini Kit (QIAGEN)

ViroGene Viral RNA/DNA Extraction Kit (ViroGene)

If using a spin column-based sample preparation procedure including washing buffers containing ethanol, an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid, is highly recommended.

E. Protocol

- The overall protocol of the analysis consists of the following main workflow:

1. Sample Preparation
2. RNA Preparation
3. Reaction Setup, Reverse Transcription and Amplification (Real-Time RT-PCR)
4. Data Analysis – Validity and Qualitative Result

- We recommend proceeding through the protocol without interruption to avoid potential degradation of the processed samples and reagents. If necessary, you may store the final RNA preparation at ≤ -18 °C until further processing. Avoid repeated freezing and thawing of the RNA preparations.

1. Sample Preparation

- accepted sample types include: Serum, EDTA and Citrate .

2. RNA Preparation

a) ViroGene® RNA/DNA Purification products

- All kinds of sample matrices Serum and Plasma may be processed with ViroGene® Viral RNA/DNA Purification products
- For Internal Control, please add Internal Control Directly to Lysis Buffer in Extraction in order of 1/10 of elution volume (if elution volume is 100 ul please add 10 ul of Internal Control per sample).

Reaction Setup and Amplification (Real-Time RT-PCR)

- Pipette 15 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the control (Quantification Standard, Positive or Negative Control).
- For quantification purposes all Quantification Standards (QS1 to QS4) should be used.
- Thoroughly mix the samples and controls with the Master Mix by up and down pipetting.
- Close the 96-well reaction plate with an appropriate optical adhesive film and the reaction tubes with appropriate lids.
- Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~3000 rpm).

Number of Reactions (rxns)	1 SAMPLE
RNA Buffer Mix	12.5 µl
Primer and Probe Mix	1.5 µl
RT Enzyme Mix	1.0 µl
Volume Reaction-Mix	**15.0 ul**

- Place the cavities in the Real-Time PCR thermal cycler and run the test with ViroGene® Profile I as given below.

Thermal Profile				
Step No	**Description**	**Temperature**	**Duration**	
1	Reverse Transcription	50 °C	20 min	
2	Activation of Polymerase	95 °C	10 min	
3	Denaturation	95 °C	15 sec	
4	Annealing & Extension	55 °C	1 min	50 cycles
5	Fluorescence Detection	channels FAM and HEX		

- ViroGene® Thermal profile allow for combined run of this and most other ViroGene® RT-qPCR detection methods as well as ViroGene® PCR detection products.

- In the event of a combined Real-Time (RT-)PCR run, make sure all necessary channels are detected.

- Please follow the specified instructions of your Real-Time PCR thermal cycler as recommended by the manufacturer.

4. Data Analysis – Validity, Qualitative and Quantitative Result

General

- The amplification data can be processed automatically using the specific software tool of your Real-Time PCR thermal cycler. Alternatively, the threshold can be set manually considering the following directions: The threshold should cross the FAM and HEX curves in the exponential phase of the PCR reaction (best visible as a linear slope when the Y-scaling is set to logarithmic). By setting the threshold, the crossing points with the HEX- and FAM-curves determine the respective cycle threshold (Ct), which is negatively correlated with the initial concentration of copies of the target genes in the Real-Time RT-PCR reaction.

- Only curves with the typical exponential amplification, meaning the curve of the raw data shows a flat baseline at the beginning, followed by a clear exponential phase and optionally reaching a plateau phase should be regarded positive.

- The actual test analysis starts with the validity check of the entire Real-Time RT-PCR run. Afterwards, by means of the Internal Control the validity of each sample reaction and its true

test result can be verified according to the Ct-value of the Internal Control channel (HEX). Finally, the HCV-specific status of each sample is analyzed (FAM).

Test Evaluation

- The **Real-Time RT-PCR test run** is only **valid,** if the FAM-curve and HEX-curve of the Negative Control are negative (Ct > 35) and the FAM-curve and HEX-curve of the Positive Control are positive. For a valid test the FAM-Ct-value and HEX Ct-value of the Positive Control have to be > 15 and ≤ 35.

- If one or more of a RNA Isolation Control (RIC(s)) is processed, its FAM- and HEX-curves must be negative.

Target	Channel	Signal		
Internal Control	HEX	positive	positive / negative	negative
HCV	FAM	negative	positive	negative
The sample is HCV		**negative**	**positive**	**inhibited**

Quantification of HCV specific RNA can be archived through external quantification standards QS1, QS2, QS3 and QS4 supplied with the ViroGene HCV RT-PCR Kit. The standards were calibrated using the WHO international standard for Hepatitis C Virus RNA. The concentration of the Quantification Standards is given in International Units (IU) per ml, corresponding to the concentration of the purified nucleic acid.

- For the validity of a quantitative diagnostic test run, all control conditions of a valid qualitative diagnostic test run must be met. Furthermore, for accurate quantification results a valid standard curve has to be generated. For a valid quantitative diagnostic test run, the following control parameter values of the standard curve should be achieved

Control Parameter	Valid Value
Slope	**- 3.00 / - 3.74**
PCR Efficiency	**85%/115%**
R square (R2)	**> 0.98**

5. Product Limitations

- Users must be trained and familiar with this product and procedures prior to application.
- Results generated by this product must be interpreted in context to clinical or further laboratory findings. It is the user's responsibility to verify performance for any procedures applied in their laboratory that are not covered by the performance data shown below.
- A negative result does not confirm status of non-infection, as results depend on appropriate specimen collection, viral load in specimen above LOD and absence of inhibitors, which would lead to invalid results. By use of the Internal Control, this product allows testing for the presence of PCR inhibitors and consequently minimizes the risk of false negative results.

F. Performance Characteristics

1. Analytical Sensitivity

- The Limit of Detection is < 15 IU/ml of RNA eluate using an in-vitro transcribed RNA specific for target gene of HCV

2. Analytical Specificity

2.1 Inclusivity

- Determination of the epidemiological sensitivity is based on *in silico* testing using the nucleotide sequence database NCBI (National Center for Biotechnology Information). Oligonucleotide sequences (primers and probe) of the method were tested theoretically with regard to a cross-reaction or unspecific hybridization. Therefore, the oligonucleotide sequences were aligned with the non-redundant data bank for DNA and RNA ("GenBank", NCBI) using Primer-BLAST and NBLAST.

1.1 Exclusivity

- The analytical specificity of the ViroGene Quantitative HCV RT-PCR kit is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligo-nucleotides were checked by sequence comparison analysis against public available sequences to ensure that all relevant HCV genotypes will be detected.

Over a hundred different HCV negative plasma specimens were analyzed with the ViroGene Quantitative HCV RT-PCR kit None of these showed a positive HCV specific signal. But all showed a valid IC signal.

In addition, the specificity of the ViroGene Quantitative HCV RT-PCR kit was evaluated by testing a panel of genomic DNA/RNA extracted from other Hepatitis or other pathogens significant in immunocompromised patients.

- Furthermore, both assays combined in the ViroGene® HCV kit were analyzed in-silico for cross-reaction with other viruses using the nucleotide sequence database of the NCBI (National Center for Biotechnology). Therefor the oligonucleotide sequences were tested theoretically with regard to cross-reaction and unspecific hybridization.

3. Reproducibility

- To test the Intraassay Variance samples with defined CT-values were analyzed in replicates in the same PCR run. The standard deviation of the replicates is < 0.5 CT values, the correlation coefficient is < 5 %.

4. Robustness

- To test the Intraassay Variance samples with defined CT-values were analyzed on different machines using different operators. The standard deviation of the samples is < 0.5 CT values, the correlation coefficient is < 5 %.

G. Symbols used on labels

Product number

Batch code


Contains sufficient for “n” tests/reactions (rxns)


Temperature limitation


Version


Use until


Caution


Consult instructions for use


Manufacture